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RESTORATION OF VISION AFTER CROSSING OF OPTIC NERVES AND AFTER CONTRALATERAL TRANSPLANTATION OF EYE

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IN REGENERATION of the optic nerve in amphibians the ingrowing optic axons, despite extreme intertangling and disarrangement of their original intraneural pattern in the scar region, manage somehow to restore their former functional associations in the brain centers in a discriminate systematic manner. Central synaptic connections are selectively reestablished by the various regenerating fibers in accordance with the specific location of their ganglion cell bodies in the retinal field. This orderly restoration of central reflex associations is predetermined in the regeneration process itself and is not aided by, nor subject to, any functional readaptation process such as learning.

The regulative growth phenomena which cause the central reflex relations to be laid down in a systematic way are not known, of course, in any concrete detail. However, logic demands the conclusion that the regenerating optic axons from different retinal loci possess differential properties of some sort by which they are distinguished from each other in the centers. It has been presumed that this specification of the optic fibers arises embryologically through a field differentiation of the retina. Peripheral differentiation is thus construed to have, in the visual system as well as in the spinal system (4, 6) an important organizing influence on the formation of central synaptic connections. The possibility has been pointed out that the type of growth-regulating factors responsible for this precise patterning of central synaptic associations in optic nerve regeneration may well be the same as those which regulate the systematic prefunctional development of reflex relations in ontogeny.

The foregoing conclusions and correlated conjecture are based primarily upon the results of experiments in which optic nerve regeneration has been combined with a 180° rotation of the eye on its optic axis (5, 6, 7). The present experiments in which optic nerve regeneration is combined with cross union of the optic nerves on the one hand and with contralateral transplantation of the eye on the other were undertaken to extend the evidence on visual recovery in amphibians and to check further the explanation given above. Optic nerve crossing and contralateral eye transplantation both produce new combinations between visual centers and periphery sufficiently different from those created by eye rotation to make it important to find out if recovery of vision under these new conditions would conform to the above explanation or necessitate its modification.

Cross Union of Optic Nerves

METHOD AND MATERIALS

Reciprocal end-to-end crossing of the optic nerves was performed in 22 anuran amphibians (9 *Hyla crucifer*, 4 *H. femoralis*, 2 *H. gratiosa*, 2 *H. cinerea*, 2 *Rana grylio*, 1 *R. sphenocephala*, and 2 *Bufo terrestris*). Except for one *R. grylio* which was only half grown, all these animals were full-size adults. The optic chiasma was exposed through the roof of the mouth and the chiasma proper was excised leaving four optic tract stumps, two on each side. The cut end of the prechiasmatic stump on each side was then apposed to the end of the postchiasmatic stump of the same side. Because natural crossing of fibers at the chiasma is complete in the amphibians (11), the above procedure resulted in a reciprocal cross connection of the right and left optic tracts. The nerve stumps were freed and displaced dorsally along the lateral wall of the brain, when apposed, to prevent their growing ventrally and crossing at the original chiasma. The regenerating optic fibers of each eye were thereby forced to grow centrally into the ipsilateral optic lobe instead of into the contralateral optic lobe as under normal conditions. All operations were performed with the aid of a dissecting microscope while the animals were under ether anesthesia. After operation the animals were kept at or a few degrees below room temperature in groups of five or less in 7-liter aquaria and terraria.

The criteria used for testing the nature of visual recovery included (i) imposed optokinetic reactions elicited by a large striped drum rotated around the animals on 3 different axes perpendicular to each other, namely, the dorsoventral, rostrocaudal, and dextrosinistral axes of the body; (ii) localizing responses consisting of attempts to approach and capture a small lure object such as a housefly impaled on the end of a fine wire and presented at distances from 5 to 15 cm. in various sectors of the visual field in random order or moved about slowly from one sector of the field to another; (iii) spontaneous optokinetic movements of head and body on (a) the dorsoventral body axis and on (b) the rostrocaudal body axis; (iv) escape reactions at the approach of a large object. These visuomotor reactions served as indices of the differential spatial values or "local signs" restored to the retinal areas by optic nerve regeneration. Care was taken to avoid observational errors which might be caused by sensory cues other than visual or by reflection and refraction of light from glass-walled containers.

Results

The first signs of recovery of vision appeared as early as the 12th day after operation in three of the group of *H. crucifer* and had appeared in eight of these animals by the 15th day. In the other species recovery was considerably slower, taking as long as 48 days in a full-grown southern bullfrog (*R. grylio*) and averaging about 22 days in the other animals. Visual recovery failed to occur in one animal (*H. crucifer*) and occurred on only one side in 2 animals (*H. crucifer* and *H. femoralis*). These failures were apparently due, so far as could be determined from histological examination, to poor apposition of the nerve stumps and consequent failure of the outgrowing fibers to reach the degenerated central nerve tracts. In all other cases the recovery of vision as indicated by the criteria employed was good and its character clear-cut.

Imposed optokinetic reactions. Optokinetic responses to rotation of a striped drum around the dorsoventral axis and also around the rostrocaudal axis of the body were made in the reverse direction from those made by normal animals (for a description of the normal responses see [1]), *i.e.*, the slow pursuit phase of head movement occurred in the direction opposite to that of drum rotation and the rapid phase in the same direction as drum rotation. In animals with vision in only one eye—either because the other eye had not yet recovered or because the other optic nerve had purposely

been severed—the reversed responses to horizontal movement of the visual field were stronger toward the seeing side as would be expected in view of the type of unilateral control which commonly predominates in these reflexes. However, reversed responses to the blind side also could be elicited to some extent in nearly all cases, and sometimes these were quite pronounced.

In contrast to the optokinetic reactions elicited by rotation of the visual field around either the dorsoventral or rostrocaudal body axes, those elicited by rotation of the visual field around the dextrosinistral body axis were not reversed but normal in character. The head was elevated or lowered correctly with the visual field as it passed upward or downward in front of the eyes.

Spontaneous optokinetic movements. Self-initiated circus movements were a conspicuous feature of the results. One of the earliest signs of recovery, they first appeared as slow swaying movements of the head to one side followed by an abrupt return movement quite like the imposed optokinetic reaction except that they were initiated without any actual movement of the visual field. They became more pronounced as regeneration was completed until many of the animals characteristically kept turning the entire body around and around in the same spot whenever aroused. In animals with both eyes functioning these circus movements were made either to right or to left depending apparently on the direction in which the initial head movement was made. The direction of head and body rotation could be controlled to some extent by jerking the animals quickly to one side so as to elicit a compensatory vestibular movement of the head, after which there tended to be a rebound movement in the opposite direction which was continued and reinforced by visual stimuli. Animals with only one eye functioning showed circus movements predominantly toward the seeing side, but as with the imposed optokinetic responses, the unilaterality was not complete, and reactions toward the blind side also were observed.

In addition to these circus movements to the right or left, the animals displayed turning movements of the head on its rostrocaudal axis. With the tip of the nose almost stationary the head was gradually tilted so that one eye was raised dorsally while the other was lowered ventrally. Pure rotation of the head on its rostrocaudal axis in this manner was occasionally observed but it was usually combined with horizontal turning in varying degree. Optokinetic movements about the rostrocaudal axis were most pronounced in the *H. cinera* and *H. crucifer*.

Similar spontaneous optokinetic reactions had also been observed previously in a few anurans after 180° rotation of the eye (7), but their occurrence in the present cases, for reasons not yet clear, was decidedly more marked and more consistent from animal to animal. The imposed optokinetic reactions merged into spontaneous responses when the animals were inside the rotary drum. Both the reversed, imposed optokinetic reactions and the spontaneous optokinetic movements tended to be exaggerated over the normal optokinetic response.

Eye movements. The reversed optokinetic responses and the spontaneous

circus and rotatory movements of head and body were both accompanied by optic nystagmic movements of the eyes themselves in their sockets. The eye movements were coordinated with the head movement in normal manner so as to follow the apparent direction of movement of the visual field or rotating drum. Conjugate movement of the two eyes persisted after one optic nerve had been severed. Sometimes the head was turned through only part of its range or remained almost stationary while the eyes continued to make full-range movements. The eye movements were most conspicuous in *R. grylio* in which they were observed before as well as after operation. They were easily followed too in *B. terrestris* and, though less pronounced, were distinctly discernible in *H. crucifer*, *H. cinerea*, and *H. gratiosa*. The movements of the eyeball can be studied much more closely and conveniently during the spontaneous circus movements than during imposed optokinetic reactions because the animals do not have to be placed inside a revolving cylinder. Special mention is made of these eye movements merely to correct the impression (11) that they do not occur in amphibians.

Spatial localization of small objects. Localization of small objects in space, as tested with a housefly impaled on a fine wire, was quite accurate when the lure was presented in the visual field exactly in the midsagittal plane. At all other positions, however, the lure was erroneously localized at a corresponding point on the opposite side of the mid-plane. These errors of localization were quite precise. When the lure was held close to the mid-sagittal plane in front of the animals, they struck close to the lure but on the opposite side of the midline. When the lure was held at increasing distances away from the mid-plane, the errors were correspondingly greater to the opposite side. Also the direction of error could be consistently and alternately reversed by presenting the lure successively first on one side and then on the other. These errors of spatial localization were best demonstrated in the toads because they struck with a flick of the tongue with little or no shift of body position. The leaping of the frogs along with their spontaneous circus movements made detailed estimations of the degree of error less easy but with repeated testing the same type of error in spatial localization was clearly demonstrable.

Escape reactions. When the animals were excited and purposely allowed to escape from their containers, they were inclined to try to jump away from any approaching large object. When the experimenter's hand was extended toward the animal's head from one side and from a little in front under such conditions, normal control frogs turned at an angle and jumped off in the opposite direction. When the hand was similarly extended toward animals with the optic nerves crossed, however, they turned toward the approaching hand and frequently leaped directly into it.

Effect of experience. The present experiments were concerned with the reestablishment by regeneration of retino-central reflex relations and no thorough measures were undertaken to find out if the regenerated connections after they had once been formed could subsequently be made function-

ally adaptive by practice and learning. After recovery the animals' circus movements were noted and their localizing reactions were tested at each feeding at three-day intervals but there was no attempt to administer the tests in such a way as to make them conducive to learning. The following observations bearing on the problem of functional readjustment are therefore only suggestive, not decisive.

The majority of cases were retained longer than two months after recovery of vision; four were kept longer than four months; and one as long as five and one half months. The reversed optokinetic reactions, spontaneous circus responses, and erroneous spatial localization of small objects were all present up to the time of sacrifice. There was no positive correction in any case of any of the reversed reactions. Most of the motor signs of disoriented vision remained sufficiently pronounced to make it difficult to say whether or not there had even been any inhibition of the maladaptive responses. The spontaneous turning and twisting of the head and body seemed to be somewhat lessened after two months in some of the animals, but it could not be certain whether this was due to specific inhibition of visuomotor reflexes or to a general depression of excitation due to prolonged laboratory confinement and handling. In the case of avoidance and escape reactions it was clear that the animals no longer jumped directly into the experimenter's hand at the end of the experiments as readily as they had done at first. They were inclined at the end not to try to jump at all under the test conditions but when they did respond, they still leaped to the side of, rather than away from, approaching large objects. There was no inhibition of the erroneous predatory pursuit and striking responses.

HISTOLOGY

Microscopic examination of the crossed optic nerves sectioned at 10μ and prepared by the activated protargol method of Bodian revealed that the optic tracts had regenerated to the ipsilateral optic lobe as intended and as indicated by the functional tests. In most cases a few fibers had strayed out from the nerve scar into the nearby white matter of the diencephalon and in a few instances it appeared that some of the stray fibers had crossed to the contralateral side in the region previously occupied by the optic chiasma. There had been no indication from the functional tests that any of these stray fibers had succeeded in forming functional connections. In apposing the nerve stumps in these experiments it was necessary that the cross sections meet in an inverted position. The actual extent of inversion of the fiber pattern, however, would depend upon the amount of fiber rearrangement in the excised portion of the optic tract at the chiasma region. In any case the fibers intertwined in the nerve scar in a typical disorderly manner without any clear indication of an orderly inverted decussation to correct for the inverted orientation of the nerve ends.

CONTRALATERAL EYE TRANSPLANTATION

The interest in contralateral eye transplantation for the present experiments lies in the fact that the eyeball cannot be shifted from the orbit on one side of the head across to the other without reversing it on one of its axes. If the transplanted eye be correctly oriented on its optic axis, then it may also be correctly oriented on either, but not both, the dorsoventral or the nasotemporal axis. If one of these is correct, the other will be inverted. Any axis of the eye perpendicular to the optic axis may be correctly oriented along

with the optic axis, but always a third axis perpendicular to both of these will be inverted. Hence, if recovery were to conform with the previous results, it follows that the appearance of the visual field should be spatially inverted on one cross dimension. Inversion with respect to one dimension only is to be distinguished from the complete inversion in all cross dimensions produced by 180° rotation of the retinal field.

METHOD AND MATERIALS

Contralateral transplantation of the eye was carried out in 21 full grown adult urodele amphibians (15 *Triturus viridescens symmetricus*, 6 *Triturus v. viridescens*) and in 30 anuran tadpoles (23 *R. clamitans*, 1 *R. pipiens*, 6 *R. grylio*). Eye transplantation in the urodeles was performed by simply cutting around the eyeball, freeing it of all its connections in the orbit including the optic nerve, and reimplanting it in the freshly denuded contralateral orbit with the optic axis properly oriented. In most of the tadpoles the eye was freed, extirpated, and reinserted through an incision in the roof of the orbit, leaving the external cornea intact. In a few of the older tadpoles in which the internal and external larval layers of the cornea had already become well fused, a circular incision around the cornea was used as in the urodeles. The eye transplantations were unilateral only.

Some of the animals were placed for the first few days after operation in a refrigerator at a temperature of about 10 degrees C., but others which were left at room temperature without the cold treatment apparently recovered just as well. The animals were kept in groups of about five in 7-liter aquaria and terraria. The criteria of visual recovery were similar in the newts to those described previously (5, 6) and in the metamorphosed frogs to those used in the foregoing experiments except that avoidance reactions were not used.

RESULTS

Recovery in anurans

Attempts to obtain functional recovery after eye transplantation or even after direct reimplantation in anuran amphibians have not led to much success in the past. On the basis of his own and previous experiments, Keeler (3) expressed doubt that any appreciable vision had ever been recovered after eye transplantation in adult frogs. Even after reimplantation in larval stages in *R. pipiens* and *R. clamitans*, Stone (8, 9) failed to get a single recovery out of 92 cases and he concluded that vision is never recovered in these anuran grafts because the eyes do not possess the power to regenerate a new optic nerve.

Subsequent work, however, has shown that the optic nerve of the tadpole and even of the adult regenerate readily to functional completion under other conditions (7) and hence some further exploratory efforts to obtain visual recovery after eye transplantation seemed to be indicated. In the present experiments eye transplantation was first tried in 14 tadpoles of various mid-larval stages. All the eyes underwent initial degenerative changes, but eight of them had regained approximately a normal external appearance by the time of metamorphosis. Consistent with Stone's results, however, there was no indication of visual recovery in any of these animals.

Contralateral eye transplantation was then repeated in 16 metamorphosing tadpoles in which the eyes were well advanced in their rapid metamorphic enlargement to adult proportions. The range of stages at which this second group was operated on centered about the time of emergence of the fore-

limbs. At this stage of metamorphosis the walls of the enlarging eyeball become extremely soft and are easily torn and punctured, but it was thought that the same rapid growth processes which tended to make transplantation more difficult would at the same time enhance conditions favoring functional recovery. This was borne out, since, in contrast to the previous results of eye transplantation either in earlier larval or in adult stages, the transplanted eyes in this group in all but two cases healed nicely without loss of size, clouding of the lens or cornea, or other signs of degeneration. Moreover, in two animals, excellent visual recovery was obtained. It seems likely that recovery of vision under these circumstances depends largely upon good approximation of the central and peripheral nerve stumps. By operating at this stage and by taking measures to insure good apposition of the nerve ends, instead of leaving their positions to chance as was done in these exploratory experiments, it might be possible to obtain a considerably higher percentage of successful recoveries.

In the two animals which recovered vision (both *R. clamitans*) the first definite signs of recovery were noticed on the 41st and 46th days after operation respectively and may have been present earlier. These consisted of optokinetic responses in the first case and of spontaneous swaying movements of the head in the second. By this time metamorphosis had been completed.

Visuomotor responses. In the first frog, the transplanted eye after recovery was oriented almost correctly on its nasotemporal axis with the dorsoventral axis inverted as illustrated in Fig. 1. As would be predicted from the orientation of the eye, the optokinetic reactions elicited by horizontal rotation of the visual field around the dorsoventral body axis were made in the correct direction. Optokinetic reactions to rotation of the visual field around the rostrocaudal body axis, however, were made in reverse. Reversed also were the optokinetic reactions to rotation of the visual field on the dextrosinistral body axis. Spatial localization of small objects was correct only for objects in or near that plane of the visual field which is determined by the optic and nasotemporal axes of the eye. The frog leaped accurately for the lure when it was held forward and in this plane. But when the lure was presented forward and above this plane, the frog struck forward and downward at a corresponding point below the mid-plane. On the other hand when the lure was presented forward and well below eye level, the animal leaped high in the air over the lure. Other pursuit and striking responses were also correctly directed with respect to the nasotemporal dimensions of the visual field but reversed with respect to the dorsoventral dimensions corresponding with the anatomical inversion of the retina.

In the second animal that recovered vision the eye had accidentally become oriented in such a position that the inherent dorsoventral axis was displaced only 90° with the ventral nib of the pupil pointed posteriorly. The original nasal pole of the eye globe was dorsal and the original temporal pole ventral. This was one of only 2 animals of the group in which a marked deviation from the intended placement had occurred. Because this acciden-

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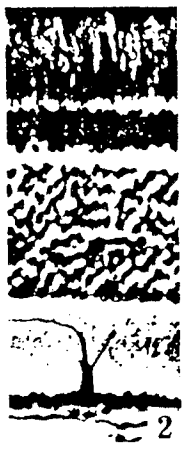

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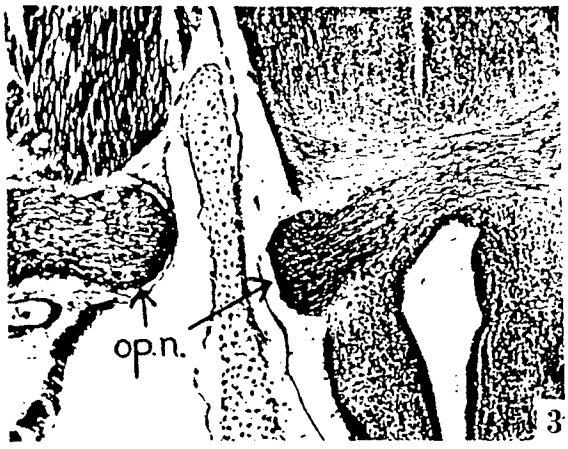

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External appearance and sectional views of retina and regenerated optic nerve of frog eyes in which there was recovery of vision after contralateral transplantation. Fig. 1 and 2 from first case and Fig. 3, 4 and 5 from second case described in text.

FIG. 1. Inversion of dorsoventral axis of the eye shown by dorsal position of pupillary nib, 3½ months after transplantation.

FIG. 2. Radial section through peripheral portion of retina. Ganglion cell layer with one large neuron selectively impregnated is toward bottom. ×260.

FIG. 3. Regenerated optic nerve shown approaching from left and entering brain at optic chiasma. Optic nerve of other side had degenerated 3 months previously. In this and following figures plane of section is frontal and top is posterior. ×60.

FIG. 4 and 5. Sections through more ventral levels of chiasma with magnification increased to 197 and 372 times respectively, Note the interlacing, non-parallel course of optic fibers in chiasma, also that all fibers cross over to contralateral side except a few in most ventral regions as shown in Fig. 5.

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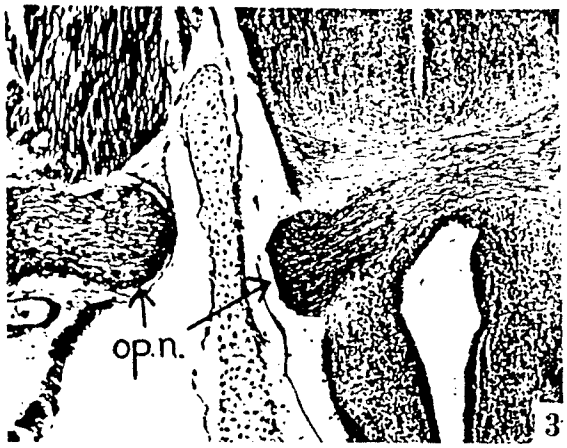

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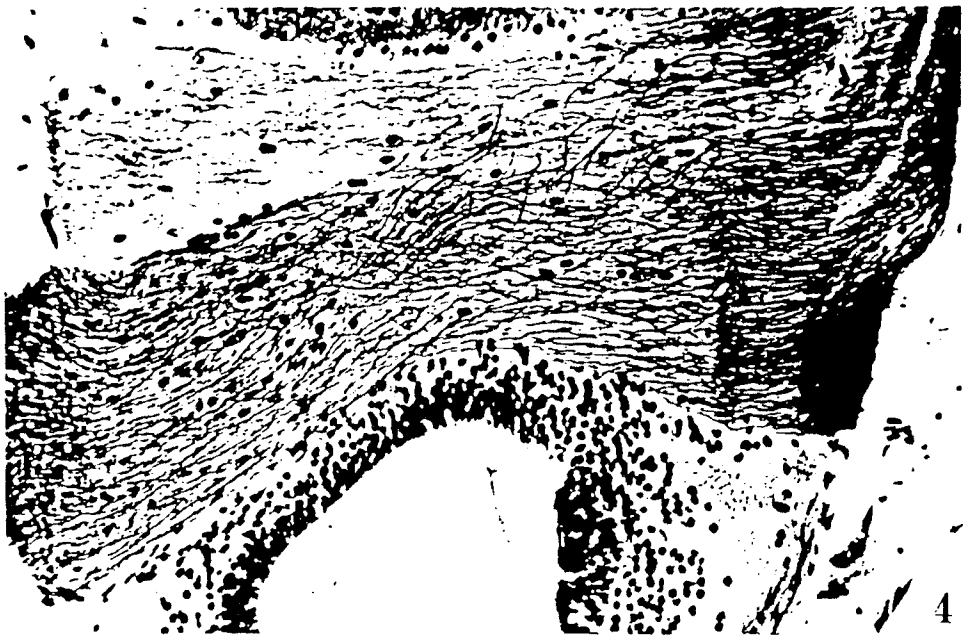

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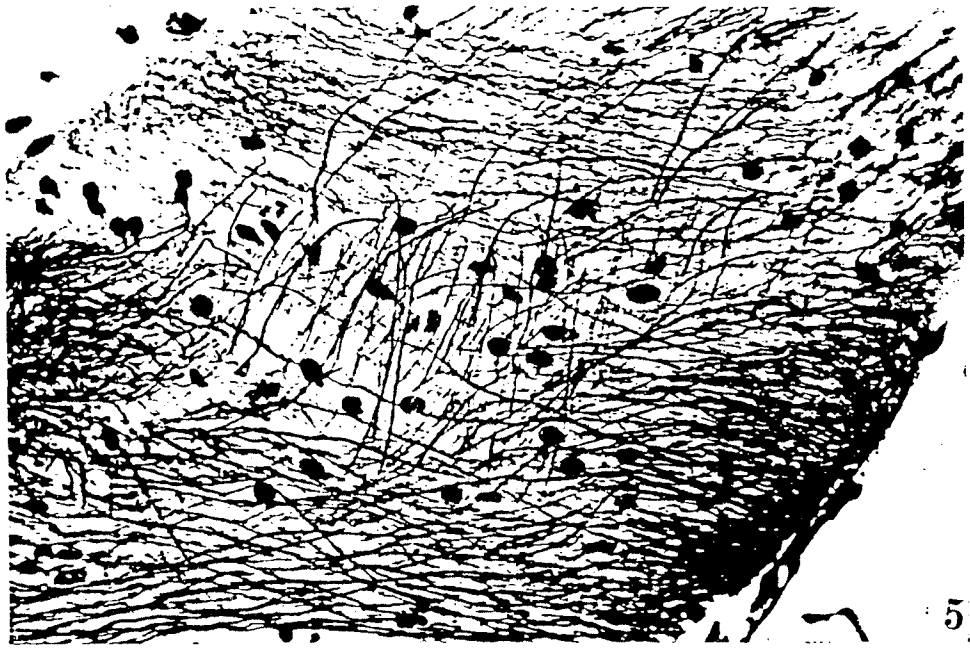

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(See page 22 for legend.)

tal displacement presented a new variation in the experiments on retinal disorientation, the correlated visual reactions are listed separately in some detail.

Rotation of the visual field around the dextrosinistral body axis of this second animal caused reversed optokinetic responses. The head was raised as the visual field moved downward in front of the eyes and the head was lowered as the visual field moved upward. Rotation of the visual field around the rostrocaudal body axis elicited horizontal movements of the head on its dorsoventral axis. The head turned toward the seeing side when the visual field moved downward on that side and toward the blind side when the visual field moved upward. Rotation of the visual field around the dorsoventral body axis caused tilting movements of the head on its rostrocaudal axis. When the visual field moved toward the blind side, the blind side of the head was lowered and the seeing side was elevated. The opposite movement occurred when the visual field moved toward the seeing side. Often this sideward tilting of the head on its long axis was not pure but was accompanied by up-down or horizontal movements. For example, when the blind side of the head was raised, the nose was also lifted to some extent and vice versa.

When a small lure object was presented at a 45° angle anterodorsally above and in front of the head, the frog struck accurately and caught it. Its responses were correctly oriented to the lure when it was presented anywhere else throughout that plane of the visual field passing through the optic axis and the anterodorsal pole of the eye.

When the lure was presented straight in front of the animal, it tilted its head upward and, vice versa, when the lure was presented directly dorsal to the eye, this frog struck straight forward 90° in error in both instances. When the lure was presented posterior to the eye the frog struck ventrally underneath the head on the seeing side, also 90° in error.

The responses were directly reversed 180° in error with respect to that plane of the visual field determined by the optic axis and the posterodorsal pole of the eye. Thus when the lure was presented posterodorsally the animal struck anteroventrally. The second frog responded, in other words, as if its visual field appeared inverted on the plane passing obliquely at 45° anterodorsally and posteroventrally through the optic axis.

In summary the visuomotor coordinations of both these animals were as would be predicted on the assumption that the intrinsic functional relations between the different retinal points and the new contralateral brain centers were restored in their regular pattern despite the adverse orientation of the retina and the consequent maladaptive functional effect for the organism.

Recovery in urodeles

Because it had been questionable at the start of the experiments that vision could be recovered after eye transplantation in the anuran amphibians eye transplantations were also performed at the same time on a group of

urodele amphibians, in which animal it has been shown (10) that recovery of vision can be expected with assurance in a majority of cases. Of the 21 animals operated on twelve (8 *T. v. symmetricus* and 4 *T. v. viridescens*) recovered vision out of sixteen which survived the regeneration period. In these animals the external signs of degeneration and subsequent regeneration of the various eye elements were typical of those described by Stone and Zaur (10). The first definite signs of visual recovery, however, appeared as early as the 49th and 47th days respectively in the two species and ten of the 12 cases had recovered vision by the 62nd day. This unusually short recovery time compared to those reported by Stone and Zaur may have been due to higher room temperatures or to the use of optokinetic reflexes instead of localizing responses as tests of recovery.

Visuomotor responses. Eight of the urodeles which recovered vision had the dorsoventral axis of the eye inverted and four had the nasotemporal axis inverted. In the former group optokinetic reactions to rotation of the visual field around the dorsoventral body axis were normal while in the latter group they were reversed. Contrariwise, optokinetic reactions to rotation of the visual field around the dextrosinistral body axis were reversed in the former group and normal in the latter group. Reactions to rotation of the visual field on the rostrocaudal body axis, if present, were not sufficiently pronounced with the apparatus employed to be recorded.

The group with the eye reversed on its nasotemporal axis showed persistent circus locomotion similar to that produced by 180 degree rotation of the eye (5, 6). There was no sign of these circus movements in the other group. The appearance of spontaneous optokinetic responses after optic nerve crossing and after contralateral eye transplantation as well as after 180° rotation of the eye lends further credence to the supposition (5) that these reactions are due to an apparent illusory movement of the visual field whenever the head is moved. Because of the rearrangements of those interrelations between kinesthetic and visual stimuli by which stability of the visual field is normally maintained despite motion of images over the retina caused by movements of eyes, head or body, the kinesthetic patterns fail to counteract the optic illusion of movement and possibly even enhance it.

In tests of spatial localization of small objects, both groups made consistent errors according to their own type of retinal disorientation. The group with inversion of the dorsoventral axis of the eye mislocated objects with respect to the dorsoventral dimensions of the visual field but located them correctly with respect to the anteroposterior dimensions. Thus objects presented above and to the rear of the eye in this group were located correctly to the rear but incorrectly below the eye instead of above. The responses indicated that the visual field appeared to these animals to be inverted about its mid-nasotemporal plane. In similar manner the group with inversion of the nasotemporal axis of the eye localized objects as if the visual field appeared to be inverted about its mid-dorsoventral plane.

Effect of experience

The two frogs with transplanted eyes were retained two and one half months after recovery of vision and four of the newts, two with dorsoventral inversion and two with nasotemporal inversion, were kept nearly two months. No sign of correction or even inhibition of the maladaptive reactions was noted in any of these animals.

HISTOLOGY

The animals' heads were sectioned at 10μ in different planes and impregnated with silver by the Bodian method. In the frogs whose eyes had been transplanted during metamorphosis but which had failed to recover vision, it was clear that no optic fiber connections whatever had been established between retina and brain. The peripheral stump of the optic nerve was sealed off with connective tissue a short distance beyond the eyeball and although it had a faint, striated, neuromatous appearance, there were no fibers stained with normal intensity leaving it questionable whether any living axons had persisted in the stump. The ganglion cell layer of the retina was definitely reduced until in the most extreme cases only a few sparsely scattered cells remained.

On the other hand in those two animals in which vision was recovered there was a large optic nerve approximately normal in diameter connecting with the optic chiasma in the usual manner. The ganglion cell layer of the retina was not noticeably reduced and the retina otherwise appeared normal. There was a scar-like neuromatous swelling in the optic nerves close to the eyeball suggesting that the old structure of the nerve stumps had not completely disintegrated as it does in the adult urodele (10). The external appearance of the eye and the histological appearance of the retina and optic nerve stump all favored the assumption that the retina had not undergone degeneration and that the optic nerve had regenerated by first intention from approximately the point where it had been sectioned, but this inference requires confirmation. Although the peripheral and central nerve stumps necessarily met each other in an inverted position there was no indication in the scar region of an orderly inversion of the fiber pattern to correct this. At the chiasma nearly all fibers crossed over contralaterally showing no tendency to double back to the ipsilateral side. In the most ventral levels of the chiasma, however, a small sprinkling of fibers turned ipsilaterally in the direction they would regularly have taken had the eye been left on the normal side. The section in which this was most conspicuous is illustrated in Fig. 5. These few stray fibers became very fine after being separated from the main optic tract bundle and they could not be traced beyond the chiasma. On the whole the fiber pattern in the chiasma offered no support for assuming that the turning of fibers to one side or the other was determined by other than mechanical factors.

As shown in Fig. 4 and 5 the optic fibers did not regenerate in straight parallel lines maintaining their original intraneural pattern but instead showed considerable interweaving and rearrangement. Some interlacing of fibers was present in the chiasma of normal control cases but was not so marked as in the two experimental animals. Beyond the chiasma the regenerated optic tract followed a regular course to the contralateral optic lobe. There was a distinct reduction in the width of the superficial layers of the optic tectum on the unregenerated side.

The histological aspects of the transplanted urodele eyes conformed in general with the descriptions of Stone and his coworkers (10). The intraneural fiber pattern of the regenerated nerves was not significantly different from that already described previously for cases in which reversed vision was restored after retinal degeneration (6).

Discussion

From the types of visuomotor coordination restored after reciprocal crossing of the optic tracts and after contralateral transplantation of the eye it is evident that the regenerating optic axons had systematically reestablished specific functional associations in the brain centers according to their respective loci of origin in the retinal field. This systematic recovery of

central reflex relations was achieved despite inversion of the cross-sectional polarity of the peripheral nerve stumps, despite disorderly outgrowth of individual optic fibers across the nerve gap, and despite extreme maladaptiveness of the resultant functional effect for the organism. These results are consistent in essential aspects with those obtained after 180° rotation of the eye and therefore confirm and extend the previous conclusions.

The recovery of vision in the above manner after transplantation of the eye in adult *Triturus* reaffirms the earlier deduction (6) that when the retina, following its degeneration, regenerates from the ciliary border, as described in the studies of Stone and his coworkers (10), there is a reestablishment of the original inherent polarity of retinal specification.

After eye transplantation in adult *Triturus* there is, according to Stone and Zaur (10), complete disintegration of both central and peripheral nerve stumps before fibers from the newly regenerated retina grow out from the bulb. It may be inferred therefore that the orderly reestablishment of organized reflex relations by growth processes is not dependent upon the ingrowing fibers finding and following any specific preformed channels to particular central points. Just as in embryonic development, individual ingrowing fibers attain particular functional asscciations in the nerve centers without being guided by pre-existent pathways.

To attain a complete differential specificity of all retinal loci the retinal field must undergo differentiation on at least two separate axes. Possibly, as in the developing limb bud (2), the anteroposterior and dorsoventral axes are determined separately in the order given. If so, one would expect that contralateral eye transplantation carried out at increasingly early embryonic stages would begin at a certain point to yield normal vision after dorsoventral inversion while continuing to yield inverted vision after nasotemporal inversion.

The results indicate further the high degree to which the complex and precisely patterned neural mechanisms subserving adaptive visuomotor coordination are dependent upon inherently predetermined rather than upon functionally acquired neural adjustments. At the same time they bring into clear focus the puzzling problem of how these intricately designed neuron associations are laid down by growth processes.

Summary

1. Intrinsically well-organized visuomotor coordinations were recovered in 21 anurans after crossing of the optic nerves and in 2 anurans and 12 urodeles after contralateral transplantation of the eye.

2. These recovered visuomotor responses, however, were schematically correlated with the anatomical rearrangements and thus were extremely maladaptive. Hence their intrinsic organization could not be ascribed to learning and must instead have been a product of the regeneration process itself.

3. The experiments extend the evidence that the reestablishment of

functional relations between retina and visual centers is strictly and systematically predetermined by growth regulating factors irrespective of functional adaptation.

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